prior to and during said grafting procedure; wherein said Fas ligand-expressing antigen presenting cells expressing an antigen specific to said graft create said immune-privileged site at the site of said grafting procedure to prevent rejection of said graft in said individual.

Please cancel claims 18 and 19.

REMARKS

The 35 U.S.C. §103(a) Rejection

Claims 1 and 3-6 were rejected under 35 U.S.C. §103(a) as being unpatentable over **Bellgrau** et al. in view of **Süss** et al. The rejection is respectfully traversed.

Bellgrau et al. teach a method of inhibiting T cell-mediated immune responses by providing a recipient animal with Fas ligand or cells expressing Fas ligand. **Bellgrau** et al. do not teach the use of antigen presenting cells to express Fas ligand in said method. **Süss** et al. disclose *in vitro* data that show Fas ligand-expressing dendritic cells induce apoptosis of $CD4^+$ T cells, resulting in the down regulation of the immune response. Applicants submit

that the combined teaching of the cited references does not teach or suggest the claimed method of the instant application.

Claim 1 is drawn to a method of inducing immune tolerance using Fas-negative antigen presenting cells that express high level of Fas ligand as a result of co-infection with AdLoxPFasL and AxCANCre adenoviruses. Neither do **Bellgrau** and **Süss** teach or suggest using Fas-negative antigen presenting cells, nor do **Bellgrau** and **Süss** teach or suggest co-infection with two adenoviruses to achieve high level of Fas ligand expression.

Conventional transfection techniques are unable to induce high level of Fas ligand expression on primary antigen presenting cells (page 41, lines 7-9). In contrast, the instant invention demonstrates high level of Fas ligand expression in almost 100% of the infected antigen presenting cells (page 10, lines 6-8; Figure 5A). The unique technique of using two adenoviruses disclosed herein allows for high titer production of adenovirus encoding the Fas ligand as well as high efficiency of infection of antigen presenting cells compared to other techniques (page 10, lines 8 to page 11, line 2). And the use of Fas-negative antigen presenting cells disclosed herein eliminates the possibility of autocrine suicide among the infected antigen presenting cells

expressing high level of Fas ligand (page 10, lines 8 to page 11, line 2). Moreover, the modified antigen presenting cells disclosed herein exhibit significantly higher functional Fas ligand activity compared to those antigen presenting cells obtained by conventional transfection methods (Figures 5B and 9). The lytic activity of the Fas ligand expressed on the antigen presenting cells of the present invention was 100-fold higher than that of transfected or activated antigen presenting cells (Example 19, Figure 9). Hence, Applicants submit that the claimed method of using the antigen presenting cells with these unique features is not taught or suggested in the cited references.

Furthermore, the cited references do not disclose any effects of Fas ligand-expressing antigen presenting cells *in vivo* as described herein. **Süss** et al. only disclose *in vitro* culture data. The differences between *in vitro* and *in vivo* situations become more important in view of the fact that **Chen** et al. (previously made of record) teach that the *in vivo* microenvironment and the presence of secondary factor *in vivo* play an important role in regulating the effect of Fas ligand expression (see abstract; page 1715, left column, lines 17-20; page 1715, middle column, lines 1-4). Thus, Applicants argue that at best, one skilled in the art might find it obvious to try

various combinations of the elements culled from these references. However, "obvious to try" is not the standard of 35 U.S.C. §103. *In re Geiger,* 815 F.2d 686, 688 (Fed. Cir 1987).

In contrast to the cited references, the present invention discloses detailed data on the effects of Fas ligand-expressing antigen presenting cells *in vivo*. Antigen presenting cells expressing high level of Fas ligand were injected into T cell receptor transgenic mice (Example 16). Applicants results showed that antigen specific proliferative responses were inhibited in mice injected with the Fas ligand-expressing antigen presenting cells, whereas there was no inhibition in transgenic mice that do not express Fas (page 42, line 3 to page 43, line 7; Figure 6). These results indicate that Fas ligand expression on antigen presenting cells bearing the specific antigen is capable of inducing T cell unresponsiveness resulted from Fas-Fas ligand interaction. Example 17 and Figure 7 further showed a rapid and profound *in vivo* clonal deletion of antigen specific T cells induced by the Fas ligand-expressing antigen presenting cells in these transgenic mice. The migration of and induction of apoptosis by the Fas ligand-expressing antigen presenting cells *in vivo* were demonstrated by histochemical and *in situ* TUNEL staining respectively (Example 18, Figure 8). The modified antigen

presenting cells of the instant invention also prolong transgene expression in liver and decrease T cell expansion in spleen as determined by immunohistochemical staining (Examples 20, 21 and Figures 10B and 11). Taken together, these results provide a detailed description of the effects of using the disclosed Fas ligand-expressing antigen presenting cells *in vivo* that is neither taught nor suggested in the cited references.

The Examiner argued that the Applicants used Fas deficient mice and *in vitro* experiment to measure apoptosis. However, as presented above and described in details in the specification, the Examiner's assertion is not correct. A series of experiments were performed to examine *in vivo* the effects of the Fas ligand-expressing antigen presenting cells (Examples 17, 18, 20, 21 and Figures 7, 8, 10B and 11). Moreover, Applicants derived antigen presenting cells from Fas deficient mice, and then injected the modified antigen presenting cells into normal mice. T cell tolerance was only induced by the antigen presenting cells disclosed herein in normal mice that express Fas, whereas no tolerance was induced in Fas deficient mice where there was no Fas-Fas ligand interaction (Example 16, Figure 6B; Example 17, Figure 7).

In view of the above remarks, the combined teaching of these two references does not provide a person having ordinary skill in this art with the requisite expectation of successfully producing Applicants' claimed method. The invention as a whole is not *prima facie* obvious to one of ordinary skill in the art at the time the invention was made. Accordingly, Applicants respectfully request that the rejection of claims 1 and 3-6 under 35 U.S.C. §103(a) be withdrawn.

Claims 1 and 3-6 were rejected under 35 U.S.C. §103(a) as being unpatentable over **Bellgrau** et al. in view of **Schuler** et al. The rejection is respectfully traversed.

Bellgrau et al. teach a method of inhibiting T cell mediated immune responses by providing a recipient animal with Fas ligand or cells expressing Fas ligand. **Bellgrau** et al. do not teach the use of antigen presenting cells to express Fas ligand in said method. **Schuler** et al. only cited the reference of **Süss** et al. (page 320, right column, paragraph 2) to suggest tolerance induction in transplantation and autoimmunity by Fas ligand-expressing dendritic cells.

Hence, the combined teaching of **Bellgrau** et al. and **Schuler** et al. is essentially the combined teaching of **Bellgrau** et al. and **Süss** et al. As discussed above, Applicants respectfully submit that **Bellgrau** and **Schuler** do not teach or suggest a method of inducing immune tolerance using Fas-negative antigen presenting cells that express high level of Fas ligand as a result of co-infection with AdLoxPFasL and AxCANCre adenoviruses, nor do **Bellgrau** and **Schuler** teach or suggest the effects of Fas ligand-expressing antigen presenting cells *in vivo* as disclosed herein. The invention as a whole is not *prima facie* obvious to one of ordinary skill in the art at the time the invention was made. Accordingly, Applicants respectfully request that the rejection of claims 1 and 3-6 under 35 U.S.C. §103(a) be withdrawn.

Claim 16 was rejected under 35 U.S.C. §103(a) as being unpatentable over **Bellgrau** et al. in view of **Süss** et al. The rejection is respectfully traversed.

Bellgrau and **Süss** have been discussed *supra*. In contrast, claim 16 is drawn to a method of creating immune-privileged sites using Fas ligand-expressing antigen presenting cells generated by co-infection with AdLoxPFasL and AxCANCre

adenoviruses. As discussed above, this co-infection is unique and significant in that it leads to high level of functional Fas ligand expression that is not attainable by conventional methods (page 10, lines 6 to page 11, line 2; Example 19; Figures 5 and 9). Applicants submit that **Bellgrau** and **Süss** do not teach or suggest a method of using antigen presenting cells generated by co-infection with two adenoviruses to achieve high level of Fas ligand expression, nor do **Bellgrau** and **Süss** teach or suggest the effects of Fas ligand-expressing antigen presenting cells *in vivo* as disclosed herein. Hence, the invention as a whole is not *prima facie* obvious to one of ordinary skill in the art at the time the invention was made. Accordingly, Applicants respectfully request that the rejection of claim 16 under 35 U.S.C. §103(a) be withdrawn.

Claim 16 was rejected under 35 U.S.C. §103(a) as being unpatentable over **Bellgrau** et al. in view of **Schuler** et al. The rejection is respectfully traversed.

Bellgrau and **Schuler** have been discussed *supra*, and they are essentially the same as **Bellgrau** and **Süss**. Therefore, as discussed above, Applicants submit that **Bellgrau** and **Schuler** do not teach or suggest a method of using antigen presenting cells

generated by co-infection with two adenoviruses to achieve high level of Fas ligand expression as claimed in claim 16, nor do **Bellgrau** and **Schuler** teach or suggest the effects of Fas ligand-expressing antigen presenting cells *in vivo* as disclosed herein. The invention as a whole is not *prima facie* obvious to one of ordinary skill in the art at the time the invention was made. Accordingly, Applicants respectfully request that the rejection of claim 16 under 35 U.S.C. §103(a) be withdrawn.

The 35 U.S.C. §112 Rejection

Claims 1, 3-6, 8, 9, 16 and 18 were rejected under 35 U.S.C. §112, first paragraph, for lack of enablement. The rejection is respectfully traversed.

The claims are drawn to methods of inducing immune tolerance using antigen presenting cells that express high level of Fas ligand as a result of co-infection with AdLoxPFasL and AxCANCre adenoviruses. The Examiner argued that the present invention is enabling for a method of inducing tolerance in C57BL6-*lpr/lpr* mice that do not express Fas, but not in normal individuals who express Fas. However, as presented above and described in details in the specification, the Examiner's assertion is not correct. The claimed

method disclosed herein induces T cell tolerance in normal mice that express Fas, not in C57BL6-*lpr/lpr* mice that do not express Fas (Example 16, Figure 6B; Example 17, Figure 7). Hence, the results from the animal model disclosed herein are applicable to human.

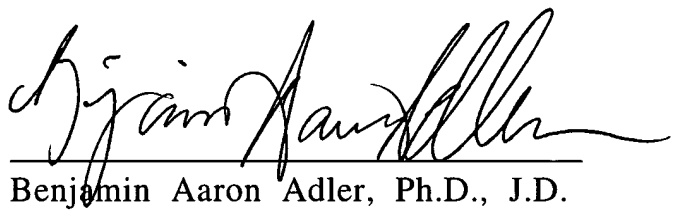
The Examiner also argued that the Applicants used *in vitro* experiments to measure apoptosis; therefore, there is no relevant *in vivo* data. However, as presented above and described in details in the specification, the Examiner's assertion is not correct. A series of experiments were performed to examine *in vivo* the effects of the Fas ligand-expressing antigen presenting cells (Examples 17, 18, 20, 21 and Figures 7, 8, 10B and 11). Hence, Applicants submit that the detailed description of the effects of the Fas ligand-expressing antigen presenting cells *in vivo* disclosed in the specification has provided sufficient enablement for using said antigen presenting cells to induce tolerance *in vivo*. Accordingly, Applicants respectfully request that the rejection of claims 1, 3-6, 8, 9, 16 and 18 under 35 U.S.C. §112, first paragraph, be withdrawn.

Claim 19 was rejected under 35 U.S.C. §112, first paragraph, for lack of enablement. However, since claim 19 has been canceled, the rejection is moot.

This is intended to be a complete response to the Office Action mailed June 19, 2000. If any issues remain outstanding, the Examiner is respectfully requested to telephone the undersigned attorney of record for immediate resolution.

Respectfully submitted,

Date: Dec 19, 2000



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